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BIOLOGICAL BULLETIN

SOME NEW TYPES OF CHROMOSOME DISTRIBUTION AND THEIR RELATION TO SEX. *Continued.*

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General.

The Chromosome Nucleoli.

The term "chromosome nucleolus" has come to mean, in a general sense, any chromosome, which remains condensed during the growth period. Under this heading, then, I shall discuss briefly the origin, valence and history of four different classes of chromosomes, namely: the accessory chromosome, idiochromosomes, m-chromosomes and differential chromosomes.

Henking ('91) observed a deeply staining body in the growth period of the primary spermatocytes of *Pyrrhocoris,* but did not detect its origin. He did recognize its chromatic nature and its unequal distribution in the second division, whereby one spermatozoön received one more chromatin element than the other. Montgomery ('98) was the first to detect that the accessory chromosome (chromosome nucleolus) was nothing more than a spermatogonial chromosome which differs in its behavior from the others, but he believed that it divided in both divisions. Paulmier, in his work on *Anasa tristis* ('99) described an accessory chromosome or what he called a "chromatin-nucleolus," which he thought originated at the time of synapsis by the union of two small spermatogonial chromosomes, divided in the first maturation division, but passed over undivided in the second. Montgomery ('01) discarded his former interpretation and accepted that of Paulmier. In the case of *Syromastes* ('04) and *Pyrrhocoris* ('06), Gross believed the accessory chromosome to arise by the fusion of two spermatogonial chromosomes which

divided in one division but not in the other. According to these interpretations, then, the accessory chromosome would be bivalent.

McClung, however ('01), maintained the accessory to be univalent. Wilson ('05 and '06) has shown in the Hemiptera heteroptera that Paulmier ('99) and Montgomery ('01) were wrong in their interpretation of the origin of the accessory chromosome, that the small bivalent divides in both divisions, and that the accessory chromosome is a univalent spermatogonial chromosome which divides in only one division, and this was confirmed by Montgomery ('06) in opposition to his earlier conclusion.

The results of Gross ('04 and '06) have been more or less of a hindrance to the correct interpretation of the accessory chromosome. Wilson ('09) again has shown that Gross was mistaken in the case of *Pyrrhocoris* and that the accessory chromosome is here univalent. He confirms, however, Gross's results on the male cells of *Syromastes.* The spermatogonial number is even (22), and the accessory chromosome is here, in fact, bivalent, but it divides in only one division. Gross figured the same number of chromosomes in both male and female cells, but Wilson inferred (not having material) that the oögonial cells would show 24 chromosomes, since the accessory chromosome is bivalent. In an addendum to his last paper ('09*b*) Wilson states that his inference has proven true and that the female cells of *Syromastes* have, in fact, 24 chromosomes. In principle, therefore, *Syromastes* conforms to those forms in which the accessory chromosome is univalent.

Another contradiction occurs in the work of Foot and Strobell ('07) on *Anasa tristis,* who assert that the densely staining body in the rest stages of the spermatocytes is a true nucleolus and not a chromosome nucleolus formed by the accessory chromosome as described by Wilson ('05 and '06) and Montgomery ('06). Wilson's reëxamination of his preparations with additional studies on smear preparations and living material, led to the same conclusion as before. Lefevre and McGill ('08) have reëxamined the form and their results agree in every detail with those of Wilson and the later ones of Montgomery. From the

evidence at hand, there seems to be no doubt that Foot and Strobell were in some manner misled.

Boring ('07), in 22 species of Hemiptera homoptera, describes the accessory chromosome as univalent and dividing only in the second division.

The work of McClung, Sinety and Sutton on the Orthoptera, has been consistent in showing that the accessory chromosome arises from a single spermatogonial chromosome. In apparent contradiction to this interpretation are the results of Voinov ('04) on *Gryllus,* Montgomery ('05) on *Syrbula* and Zweiger ('06) on *Forficula.* Each of these observers describe the accessory chromosome as arising from the fusion of two spermatogonial chromosomes and dividing in both divisions. In opposition to Montgomery's work on *Syrbula* is the recent paper by Robertson ('08) on the same genus. He seems to demonstrate conclusively that the accessory chromosome is univalent and divides in only one division. Randolph ('08) in the earwig *Anisolabis,* describes an equal pair of idiochromosomes. May it not be possible that Zweiger mistook, in *Forficula,* such a pair of idiochromosomes for a bivalent accessory?

Montgomery ('05) also described the accessory chromosome of *Lycosa,* a spider, as bivalent and arising from the union of two spermatogonial chromosomes. While he did not determine definitely the manner of its division, he was inclined to believe that it divided in both divisions, judging from the manner of its formation. About the same time Wallace ('05) published her account of *Agalena.* She figured two accessory chromosomes which divided in neither division. In an attempt to unravel these contradictions, Berry ('06) describes the behavior of the chromosomes in *Epeira.* She shows conclusively that there is an odd number of chromosomes in the spermatogonia and that the accessory chromosome is a spermatogonial chromosome which retains its identity throughout the growth period and divides in only one division. This makes the results in spiders consistent with the majority of those in the Orthoptera and Hemiptera.

The results of Blackman on *Scolopendra* ('05) and Medes on *Scutigera* ('05) show that the accessory chromosome in the Myriapoda is a single spermatogonial chromosome, but each finds

the chromosome nucleolus of the growth period ("Karyosphere") made up of all the chromosomes.

McGill in her earlier work on *Anax junius* ('04) described the accessory chromosome as arising from a union of two small chromosomes as Paulmier had done in the case of *Anasa tristis*. The later paper of Lefevre and McGill ('08) on the same form, corrects the above error and shows that the accessory chromosome is a single spermatogonial chromosome.

The accessory chromosome in the Coleoptera as described by Stevens ('05, '06 and '09) arises from a spermatogonial chromosome and divides only in one division.

The idiochromosomes as described by Wilson ('05 and '06) for the Hemiptera, Stevens ('05 and '06) and Nowlin ('06) for the Coleoptera, and Stevens ('08) for the Diptera, are two univalent spermatogonial chromosomes which usually remain condensed throughout the growth period. They unite at synapsis in the Coleoptera and one chromosome nucleolus is present during the growth period except in *Tenebrio*, where the idiochromosome bivalent does not remain condensed. In the Hemiptera and Diptera they may or may not unite at the primary synapsis to form a bivalent body. In the former case, one chromosome nucleolus is present during the growth period, but separates into its univalent elements before the first maturation division. In the latter case, two chromosome nucleoli are present in the growth period.

The m-chromosomes as described by Wilson ('05), are two spermatogonial chromosomes which do not unite at the general synaptic period and which may or may not condense in the early growth period to form two small chromosome nucleoli. They undergo a late synapsis in the prophase of the first division to form a bivalent, which divides in both divisions.

We thus see that the three classes of chromosome nucleoli—namely, those derived from the accessory chromosome, from the idiochromosomes, and from the m-chromosomes, are alike in that all are direct descendents of spermatogonial chromosomes.

The differential chromosomes or chromosome nucleoli of the Reduviidæ, which are described in the present paper, are in each case, with the possible exception of *Acholla ampliata*, univalent,

and derived from univalent spermatogonial chromosomes. The case of the large nucleolus of *Gelastocoris* is not so clear, but as previously stated, there is a possibility that the differential chromosomes are embedded in the nucleolus, although not as distinct and separate individuals as in the Reduviidæ. The differential chromosomes in the Reduviidæ, are without exception (as far as examined) embedded in a plasmosome. This plasmosome may be pale (*Prionidus, Sinea*) in which case the chromosomes stand out clearly, or it may stain rather densely (*Diplocodus, Conorhinus*) so that the chromosomes can be seen only faintly or not at all. In this respect they differ from the chromosome nucleoli formed by the accessory, m- and idiochromosomes. The accessory chromosome is, in the early part of the growth period, in contact with a pale plasmosome, but later separates from it. The idiochromosomes also may be associated during a part of the growth period with a plasmosome. The m-chromosomes may lie in any position and have no relation to the plasmosome.

Origin of the Differential Chromosomes.

In a study of the differential chromosomes, one of the most interesting questions which arises, is the origin of the asymmetrical chromosome distribution. It seems almost a certainty that these peculiar forms of distribution have not been present since the origin of the species, but have arisen sometime within its history.

In a discussion of this question, Montgomery ('06) is inclined to the belief that the m-chromosomes and idiochromosomes are not radically different structures, but are rather extremes of a series of modifications. Of their origin he says, "First a pair of autosomes (chromosomes) became modified so as to retain their compact nature during the growth period, still maintaining their approximate equivalence in volume. Because such allosomes are usually very small, we might conclude also, that they arose from the smallest pair of autosomes. In the next change would appear a growing disparity in size, which, if our last assumption be correct, would be due not to one becoming smaller and the other becoming larger, but rather to one retaining its original volume and to the other becoming much larger. This

second step would then be one of differentiation of the two, a becoming different, probably implying also difference of metabolic activities. This would account for the lessening affinity of the two as exhibited by the protraction of the time of conjugation. Then would be attained the stage of the second type of diplosomes (idiochromosomes), no longer united but separate in the first maturation spindle. And the last step would be that, instead of a reduction division of them in this spindle there would take place there an equational division of each." This conception is rendered untenable by Wilson's discovery that in *Metapodius* a typical pair of m-chromosomes and of unequal idiochromosomes coexist in the same species. In his papers of '01 and '05, Montgomery has argued that the accessory chromosome is bivalent and arose by some abnormality in mitosis as by the failure of two spermatogonial chromosomes to separate. Wilson ('05 and '06) suggests that the accessory chromosome may have arisen by the gradual disappearance of the small idiochromosome. Montgomery ('06) realizes this possibility, although he offers objections to it. While Wilson still believes that this interpretation may be applicable in many cases, in his work on *Metapodius* ('09*b*), he gives another possibility of its origin along with that of the supernumeraries. He says, "This was suggested by the observation that in a very few cases in 22 chromosome individuals, both idiochromosomes were seen passing to the same pole in the second division. The rareness of this occurrence shows that it is doubtless to be regarded in one sense as abnormal. But even a single event in an original 22-chromosome male, if the resulting spermatozoa were functional, might give the starting point for the whole series of relations observed in the genus, including the establishment of an unpaired idiochromosome. The result of such a division should be a pair of spermatozoa containing respectively 10 and 12 chromosomes. The former might give rise at once to a race having an unpaired idiochromosome and the somatic number 21 in the male. The latter might similarly produce an individual having in the first generation a single supernumerary chromosome and in succeeding generations an additional number."

In regard to the possible origin of the supernumeraries in

Diabrotica, where they are associated with an odd chromosome instead of a pair of idiochromosomes, Stevens ('08) makes the following suggestion: "It may be possible, as was surmised earlier in the study, that (1) there will prove to be two distinct types (varieties or species), in each of the present species, one having the large unpaired heterochromosome only; the other having an equal pair of chromosomes as in *Haltica,* and that (2) the irregularities in time of division and the consequent peculiarities in number and distribution of the supernumeraries in *Diabrotica* are to be attributed to hybridism."

The differential chromosomes in *Gelastocoris* and in the Reduviidæ seem to have undergone a somewhat different history from that of the other types. It is clear that the single large idiochromosome of *Diplocodus* is represented in *Fitchia* by two chromosomes, in *Prionidus* and *Sinea* by three and in *Gelastocoris* by four chromosomes. It is very probable that the condition seen in *Diplocodus* is the most primitive one, and the essential question is how the other types can have been derived from this. Taking as an example *Fitchia,* where the female number is 28 and the male 27, if a pair of idiochromosomes was the original condition in the species, the original number of chromosomes must have been 26 in both male and female. If, in the male, the original large idiochromosome should break up into two parts, two classes of spermatozoa would be found, one containing 13 chromosomes and the other 14. All the eggs would have 13 chromosomes. If an egg were fertilized by a 14-chromosome class of spermatozoa, the result would be a female with 27 chromosomes. In maturation, this female would produce two kinds of eggs, one with 14 chromosomes and the other with 13. The 14-chromosome class fertilized by a spermatozoön with 13 chromosomes would produce either a male or female with 27 chromosomes. From these individuals, then, would arise both eggs and spermatozoa with 13 and 14 chromosomes. As the 14-chromosome class of spermatozoa would be female-producing, females with 28 chromosomes would be produced upon fertilization of an egg with 14 chromosomes by a spermatozoön with 14 chromosomes. Further, an egg with 14 chromosomes, fertilized by a spermatozoön with 13 chromosomes would produce a male with

27 chromosomes. This gives the number relations as we find them at present if the individuals with the original number (26) and the females with 27 disappear. The same explanation holds for the remainder of the types if we assume that the large idiochromosome breaks up into three or four elements instead of two. Another thing which adds weight to the above interpretation is the fact that the behavior of each of the triad, tetrad and pentad groups, as a whole, is exactly comparable to the behavior of a pair of idiochromosomes. Evidently the original irregularity might have first occurred in either sex and have been transferred to the other. This gives another method by which the chromosome number may change during the history of the species. While *Metapodius* and *Diabrotica* are species where a change is actually taking place at the present time, the forms described in the present paper (with the exception of *Diplocodus*) show a fixed condition after a change has taken place.

Sex Determination.

In a preliminary note ('08) Morgan has given some interesting data in regard to sex determination in phylloxerans, where all the fertilized eggs produce females and where both males and females develop from parthenogenetic eggs. In describing the spermatogenesis he says: "The reduced number of chromosomes is three. In the first spermatocyte division two of these divide equally, but the third lags behind the others, and finally in the very last stages of this division, it retreats to one of the poles. Thus there are three chromosomes in one of the two daughter cells, and only two in the other. Still more significant is the fact that the cell with the fewer chromosomes is very small; it contains very little cytoplasm and subsequently degenerates without forming a spermatozoön. In the second spermatocyte division all three chromosomes of the larger cell divide equally, thus producing two spermatids with three chromosomes each. These spermatids become spermatozoa. They correspond in their mode of development to the 'female-producing' spermatozoa of other insects. Hence we can understand why all fertilized eggs become females."

To those who have sought to bring forward a theory of sex

determination of universal application, the parthenogenetic forms have been more or less of a stumbling block. The above results are in perfect harmony with the hypothesis of sex-production advocated by McClung, Stevens and Wilson.

Morgan also gives some further observations, indicative as to the manner in which males and sexual females are produced from parthenogenetic eggs. "I find that the somatic cells of the males of the species referred to above contain only five chromosomes. These five give in the spermatogenesis the reduced number three by two uniting with each other and the third having no partner. I find that the somatic cells of the female contain six chromosomes. It follows that at some time in the life cycle of the parthenogenetic eggs, one chromosome disappears in those eggs that become males, while the full number is retained in the female. It seems plausible that this change takes place in the formation of the single polar body given off by the parthenogenetic egg.

"The results seem to show that while the sex of the stem mother is connected with the presence of 'female-producing' spermatozoa, the production of males and of sexual females is dependent on a process that takes place in the egg analogous to the same process that takes place in the spermatogenesis of other kinds of insects. Hence it follows that the egg as well as the sperm has the power of determining sex by regulating the number of its chromosomes."

Von Baehr's recent results ('08) and also those of Stevens ('09) on the aphids confirm those of Morgan on the phylloxerans.

In a preliminary note ('08) Baltzer gives some results on echinoderm eggs, where he finds in the early cleavage stages of fertilized eggs of *Echinus microtuberculatus* and *Strongylocentrotus lividus*, an unpaired element which comes from the egg nucleus. Further, this unpaired element is present in only a part of the eggs. He suggests the possibility that this may be a chromosome which has to do with sex determination. If this element should prove to be a chromosome and in one half of the eggs, it would be the reverse of the condition found in the insects, for there the unpaired chromosome has arisen in the male. However, two such diverse phenomena are not impossible in different groups of animals, as we have no reason to suppose that

sex differentiation has taken place in the same manner in all animals. In fact, the quotation from Morgan shows that two classes of eggs are produced in phylloxerans and that the inequality of the chromosome distribution is accomplished in some manner at the time of the formation of the polar body.

McClung, Stevens and Wilson in their studies on the chromosomes of the insects have argued that the accessory chromosome and the idiochromosomes are either sex determinants or are in some manner connected with the determination of sex. The manner in which this is brought about yet remains a question. Wilson ('06) gives two interpretations by which the end result as we find it may be reached. First he follows out the earlier hypothesis of Castle ('03) that sex production may be interpreted as the result of a Mendelian segregation, transmission and dominance of the sexual characters. This interpretation involves two assumptions, neither of which have been proven, but which he argues may not be insuperable difficulties. One is the assumption that there are two kinds of eggs, the other, that selective fertilization occurs.

As an alternative point of view, he gives a second interpretation, in which he suggests that possibly the differential chromosomes may not be qualitatively different except in the degree of their special activity; or the end result may be due merely to a quantitative difference in the chromatin. Wilson, himself, in his work on *Metapodius* ('09), where the female may or may not have a larger amount of chromatin than the male, has brought forward evidence against the quantitative interpretation.

My results in the present paper offer nothing new in regard to the theory of sex determination, but are in perfect accord with the majority of previous results on the insects. I shall not venture far into theoretical discussions. There is, however, one question which naturally arises. Why do we have so few as one and two chromosomes involved in the production of males and females respectively in those forms with an odd chromosome, and as many as five and eight in *Gelastocoris?* This is not so strange, if the unequal distribution in these forms has arisen from a pair of idiochromosomes as previously explained. In every case whether the difference in number be one, two, three

or four, the female has the larger quantity of chromatin with the possible exception of *Acholla multispinosa*. Here the male seems to have the greater quantity and this inequality is brought about by the differential chromosomes themselves. This argues further against the interpretation that sex is determined by a quantitative relation of the chromatin.

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THE HYO-BRANCHIAL APPARATUS OF TYPHLOTRITON SPELÆUS STEJN.

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The cave salamander *Typhlotriton* was put in the family Desmognathidæ by Stejneger, in 1892.[1] The basis of this classification was chiefly certain skeletal characters, such as the structure of the vertebræ. The study of the hyo-branchial apparatus of this form seems to show other relationships.

In the larvæ of *Typhlotriton,* which are often found almost

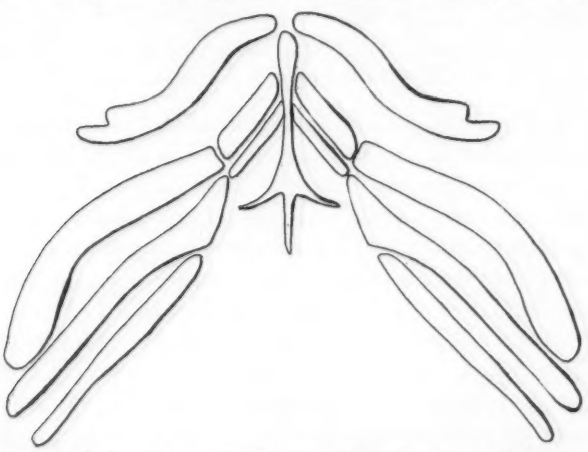

FIG. 1. Hyo-branchial apparatus of full grown larva of *Typhlotriton spelæus* from above, showing three branchial bars and a three-pointed "Copulastiel." × 6.

as large as the adult, only three branchial arches are found, Fig. 1, instead of four which are found in the larvæ of other members of the family Desmognathidæ, Fig. 2.

In the adult *Typhlotriton,* the general character of the hyo-branchial apparatus is much like the type found commonly in the

[1] Stejneger, Leonhard, "Preliminary Description of a New Genus and Species of Blind Cave Salamander from North America, *Proc. U. S. Nat. Museum,* Vol. XV., 1892.

family Plethodontidæ, especially in the character of the very long first cerato-branchial, Fig. 3.

By a comparison of the hyo-branchial apparatus of larval *Typhlotriton* with the same parts in the larvæ of the Plethodontidæ, a striking resemblance may be noticed, especially in the fact that in *Typhlotriton* as in *Spelerpes* for instance, Fig. 4, there are only three branchial bars, while the general proportions of all the parts are about the same, as the figures show.

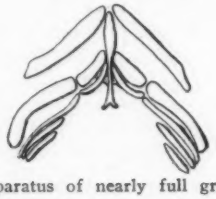

FIG. 2. Hyo-branchial apparatus of nearly full grown larva of *Desmognathus fusca* from above, showing four branchial bars. × 6.

If the hyoid apparatus of *Typhlotriton* larva be compared with that of *Typhlomolge*, another cave form, but one that has external gills, a very striking resemblance is found between the two, Fig. 5. Emerson[1] who describes the hyo-branchial apparatus of this form, suggests that it shows in many ways a marked resemblance to a *Spelerpes* larva and that it differs widely from the members of the family Proteidæ in which it has been placed.

May not this then be a larva, possibly a permanent larva as suggested by Kingsbury[2] for *Necturus?* At any rate judging from the hyoid apparatus alone there seems to be a rather close relationship between the two forms *Typhlomolge* and *Typhlotriton*.

In comparing the hyoid apparatus of *Typhlotriton* with that of larval *Spelerpes*, it may be noted that in all essential respects the two structures are alike. There is a slight difference in the proportions of parts, and the tip of the "Copulastiel" of Gaup, has three parts in *Typhlotriton* while it only has one in *Spelerpes*. The similar one of *Typhlomolge* we find divided into two.

[1] Emerson, 2d, Ellen T., "General Anatomy of *Typhlomolge rathbuni*," *Proc. Bost. Soc. Nat. Hist.*, Vol. 32, No. 3.

[2] Kingsbury, B. F., "The Rank of *Necturus* among Tailed Bacteria," BIOL. BULL., Vol. VIII., 1905.

A summary of the points in which *Typhlomolge, Typhlotriton* and *Spelerpes* agree would be somewhat as follows:

1. All have three branchial bars in the larval form. The so-called adult, but possible larva *Typhlomolge,* has three.

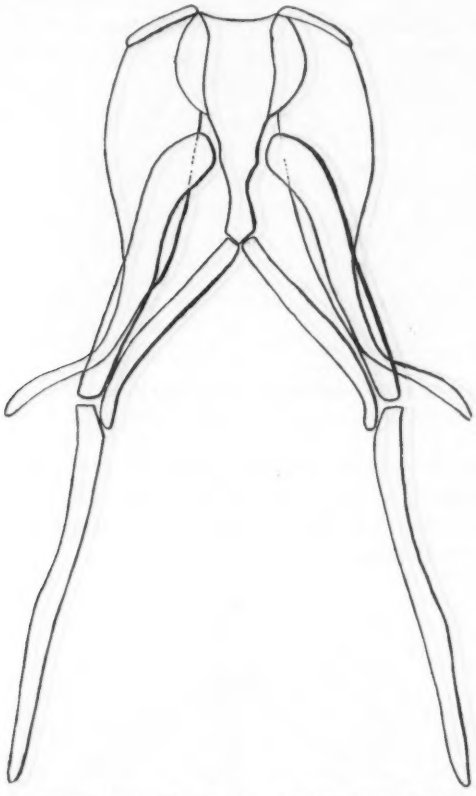

FIG. 3. Hyo-branchial apparatus of adult *Typhlotriton* from below, showing the long projecting 1st cerato-branchial. × 6.

2. The proportions and general position of the parts of the hyo-branchial apparatus are much the same in all.

3. In the adult forms of *Typhlotriton* and *Spelerpes,* the first cerato-branchial is very long.

4. The larvæ of *Typhlotriton* and *Spelerpes* grow to some size before transforming.

5. All forms live away from the light much or all of the time, two have lost the use of their eyes and numbers of the genus *Spelerpes* live in caves to some degree.

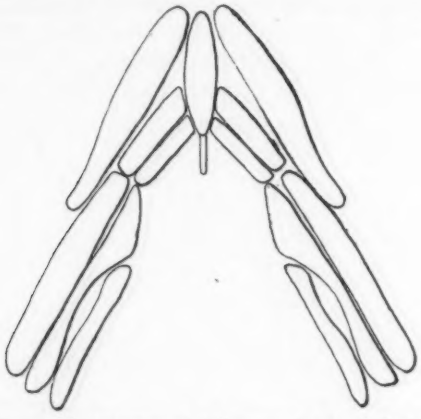

FIG. 4. Hyo-branchial apparatus of full-grown larvæ of *Spelerpes bilineatus* from below, showing the single "Copulastiel." × 6.

Judging from these similarities may not a series of forms be named in which all the members are closely related to each other, and which shows the different degrees of adaptation of one distinct line of Urodela?

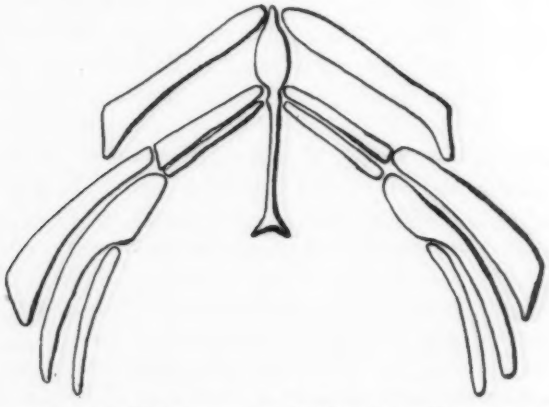

FIG. 5. Hyo-branchial apparatus of *Typhlomolge rathbuni* from above, after Emerson. × 6.

1. *Spelerpes bilineatus* which is not found in caves to any degree.

2. *Spelerpes longicauda* resembling the first form, but often called a cave salamander, although it may be found out from caves now and then.

3. *Spelerpes manculicaudus.* More properly a cave form and closely resembles the next form in many ways on the one hand, as well as *longicauda* on the other, but has well developed eyes.

4. *Typhlotriton,* found in caves. Has lost the use of its eyes and is truly a cave form.

5. *Typhlomolge rathbuni* which is found in deeper caves and seems to be a permanent larva or the larva of an adult closely related to *Typhlotriton* and *Spelerpes.*

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THE AUTOTOMY OF THE HYDRANTH OF TUBULARIA.

MAX MORSE.

When colonies of *Tubularia crocea* are brought into the laboratory, and placed in aquaria, they sooner or later lose their hydranths. The hydranths become pinched off from the stems and fall to the bottom of the aquaria where they disintegrate. Later, new hydranths are regenerated in place of the old ones.

Somewhat similar cases of the loss of the hydranth have been reported by various workers on other species. All such instances, however, involve an absorption of the hydranth, preceded by a dissolution of the body through histolysis. *Tubularia,* alone, is known to pinch off its hydranths completely from the stems before extensive histolysis occurs or indeed, any whatsoever.

Loeb[1] found in a species of *Campanularia,* an absorption of the hydranth when the individual came into contact with the sides of the dish or with any other solid object. Thacher[2] corroborated Loeb so far as an absorption occurred, but no evidence of any effect of contact in producing the action could be determined. Loeb also reported absorption in two other genera, viz., *Margelis* and *Antennularia,* where, however, the inciting cause was believed by Loeb to be the changes the stems underwent under the action of gravity. No one has studied this special feature in these forms since Loeb. As will be spoken of later, *Tubularia* does not show any such response.

Thacher (*l. c.*) described absorption in two species of *Eudendrium, E. ramosum* and *E. tenue,* and also in *Pennaria tiarella. Pennaria carolinii,* an allied species, was studied by

[1] Loeb, J., "The Transformation and Regeneration of Organs," *Amer. Journ. Phys.*, Vol. 4, 1900.

[2] Thacher, H. F., "Absorption of the Hydranth in Hydroid Polyps," BIOL. BULL., Vol. 5, p. 297.

Cerfontaine[1] and by Gast and Godlewski.[2] Cerfontaine believed that the cause of the absorption lay in the difficulty with which the fully formed hydranth responded to sudden changes in its environment, whereas the regenerated ones are better adapted by " une acclimation rapide."

Gast and Godlewski (*l. c.*) have examined the case more critically. They show that, at the beginning of the process, the tentacles are withdrawn until nothing is left externally to show where they once existed. The peristome likewise disappears and the mouth closes. Ultimately, all that is left of the hydranth is a small smooth knob. This, too, becomes absorbed into the stem and a cicatrix alone remains. Then regeneration may begin. Concomitant with these external changes, the cells of the endoderm begin to dissociate and disintegrate and come to lie as detritus in the lumen of the hydranth. The current flows down into the stem and carries the débris and this continues until the whole of the hydranth has been absorbed. The changes in the cells are not restricted to the endoderm cells for those of the ectoderm lose their vacuoles and become greatly reduced in size. They remain, however, much longer *in situ* than the cells of the endoderm. The changes seem to affect, from the first, all of the cells of the hydranth and the process is only in this sense a local one. Moreover, inasmuch as open communication between the hydranth and the rest of the stem is maintained, it cannot be urged that the process is a passive one, due simply to the death of the cells of the hydranth. In this respect, the description of Gast and Godlewski is similar to that to be given for *Tubularia*.

The first evidence of autotomy in *Tubularia* is the sinking of the hydranth upon the stem. This pendent position results from a constriction of the cœnosarc at the base of the hydranth, where it joins the stem. For a time the hydranth hangs suspended by the perisarc, but soon this breaks and the hydranth falls. During the whole of this process, including, generally, the first half hour

[1] Cerfontaine, P., " Recherches experimentale sur la regeneration et l'heteromorphose chez astroides calycularis et *Pennaria carolinii*," *Arch. de Biol.*, t. 19, 1902.

[2] Gast, R., und Godlewski, E., Jr., " Über den Regulationserscheinungen bei *Pennaria carolinii*," *Arch. für Entwickelungsmec.*, Bd. 16, 1903.

while the hydranth lies at the bottom of the dish, completely severed from the stem, the tentacles are seen to move about as in a normal individual. After a longer or shorter time, varying with external conditions, the movements cease, the hydranth becomes whitish and evidences of disintegration become apparent. The cœnosarc of the stem, on being freed from the hydranth, early shows a collection of red pigment, described by Driesch, Loeb, Stevens and Morgan in their studies of regeneration in this genus. The tube of the stem has been closed distally by the folding and compression of the walls even before the hydranth has been lost. For a time immediately after the autotomy of the hydranth has taken place, the cœnosarc withdraws for one of two millimeters down the perisarc tube, leaving free, ragged edges to the outer tube projecting beyond the protoplasmic portions. Soon, however, the cœnosarc is seen to be flush with the top of the perisarc.

Sections through hydranths and stems at the period when the hydranth is falling, show a constriction of the protoplasmic parts immediately beneath the attachment of the hydranth to the stem. The perisarc seems to take no active part in the changes involved in autotomy. The walls of the cœnosarc, involving both ectoderm and entoderm, are folded upon themselves. In some specimens there seems to be an indication of histolysis setting in among the cells forming the ectoderm and entoderm in the portion of the cœnosarc tube involved in the constriction. When it occurs, such dissociation of the cells is of local occurrence only and is to be found only in the constriction. Examination of the hydranth in such cases (Fig. 1), shows it to be perfectly normal as far as one may judge from histological evidence. Moreover, living specimens, as has been mentioned, show activity of the tentacles even after the hydranth has been removed.

For a time, varying with the individual, the hydranth remains attached to the stem by the perisarc only and it is the weight, probably, of the hydranth which ruptures the chitinous perisarc and frees the hydranth from the stem. One may see, in the individuals at this stage, resting in the aquaria, that all protoplasmic attachment has been withdrawn and longitudinal sections

bear this out. At the base of the hydranth and at the distal end of the stem, from which the hydranth has been severed, there is no definite arrangement of the cells into ectoderm and entoderm. The cells seem to lie freely in the chitinous envelop. The

FIG. 1. Sagittal section of *Tubularia* hydranth immediately after autotomy. The hydranth had fallen to the bottom of the dish.

cylindrical or flattened epithelium cells of ectoderm and entoderm of the normal individual have now assumed a spherical form. In the iron-alum stain, the nuclei appear for a time normal, but later distinct changes, undoubtedly pathological, have set in. There is no indication of an absorption of such cells into the lower portions of the stems as Gast and Godlewski (*l. c.*) have described for *Pennaria*.

The process of disintegration of the cells of the hydranth which has been cut off from the stem, is ushered in by dissociation of the cells of the entoderm (Fig. 2). There is no constancy in location of the origin of this process, but, in the main, it seems to set in at the proximal end, *i. e.*, at the end formerly connected with the stem. The entoderm cells leave their positions around the periphery of the hydranth, immediately beneath the ectoderm and come to fill the whole of the cavity of the hydranth. This cavity, at the same time, begins to become smaller in volume. Then, after a time, the ectoderm cells begin to participate in the process of dissolution. The gonanths are

FIG. 2. Hydranth which has begun to disintegrate, in sagittal section. A gonanth is shown also in section; the cells are normal.

the last to suffer (see Fig. 2). Even after the hydranth has begun to lose its shape, as it lies at the bottom of the dish, these reproductive bodies show little histolysis.

In order to determine what factors were operative in causing autotomy, the writer performed a series of experiments on *Tubularia*. The following external factors were considered: (1) Temperature effects—(*a*) heat, (*b*) cold; (2) light, involving its absence, darkness; (3) gravity; (4) aëration; (5) mechanical factors, such as abrasion, force of current, etc.

1. *Temperature.* (*a*) *Effects of Heat.*—A fingerbowl, containing a dozen stems of *Tubularia* fresh from the sea, was placed in the direct sunlight, where the temperature registered

25° C. A control was placed in the shadow near it.[1] Within half an hour evidences of autotomy became apparent and within one and one half hours, over half of the hydranths had been lost. In the control, where the temperature of the water registered 5 degrees lower (25° C.), the hydranths were retained for half a day. More striking still was the rapidity of autotomy in the case of the fingerbowl of specimens placed in an apartment immediately over the boilers of the engine-room of the Bureau of Fisheries Laboratory where the temperature is constantly at 33° C. In this case all of the hydranths had fallen entirely from the stems within two hours, while a few had fallen within one hour.

(*b*) *Cold Effects.*—A fingerbowl of specimens, 12 in number, was placed in an ice-chest at a temperature of 8° C. The hydranths in this case were retained for three weeks and undoubtedly would have persisted had not the experiment been terminated accidentally. The specimens were examined from day to day and they gave every indication of being normal, inasmuch as the tentacles were active at all times. Food was provided for the hydroids, but it was not possible to determine whether they fed or not. In another set of experiments, specimens were kept a week at a temperature of 10° C. in a fingerbowl resting in chipped ice, the bowl being placed in diffuse light. At the end of the week, the individuals were apparently healthy and no indication of autotomy could be determined.

It cannot be urged against these experiments that the animals became benumbed and their activities thereby lessened with consequent retention of the hydranths, for as we have seen, they were aparently normal and active throughout.

2. *Effect of Sunlight.*—In the experiments with the effects of light, it was necessary to insure against heat effects. Various methods were used for obviating this factor. A large vessel, with a volume of about six gallons, was provided with a platform which rested about ten centimeters from the bottom of the vessel and fifteen from the surface of the water over it. On this platform, a fingerbowl of *Tubularia* stems was placed. Then

[1] The possibility of light being the factor here is considered later.

the larger vessel was filled with water so that the fingerbowl was submerged to a depth of fifteen centimeters. The apparatus was placed in the direct sunlight, which fell upon it from eight o'clock in the morning until about four thirty in the afternoon. The temperature of the water was kept at 20° C. It was not possible to reduce the temperature of the water, under the circumstances, below this. Control specimens were placed in a similar vessel, somewhat smaller, in the shadow near the larger one. The first hydranths fell from the individuals in the larger vessel, exposed to the rays of the sun, during the third day. Those in the control gave evidence of autotomy at about the same time, or even earlier, perhaps. This result is quite similar to that given by colonies of *Tubularia* brought into the laboratory and placed in running salt water either in the light or in the dark. It seems to point to the absence of any effect of sunlight in inducing the process of autotomy. The hydranths very probably would have remained longer if the temperature could have been reduced, as is shown in other experiments to be described.

In another set of experiments fingerbowls of specimens were placed in a trough of chipped ice and set in the direct sunlight. The hydranths were retained for nearly two weeks. In a third set, under more natural conditions, stems of the hydroid were tied to weighted blocks of wood and these were submerged about fifteen centimeters beneath the surface of the water in the "outer pool" of the Bureau of Fisheries at Woods Hole, where the tide sweeps through at all times, bringing cool water from Vineyard Sound and Buzzards Bay. The individuals, of course, were subjected to the direct rays of the sun. The hydranths were retained for two weeks and this would have continued undoubtedly if the waves during a stormy period had not torn the specimens from their supports. The average daily temperature of the surface water during this time of year was 19° C. At night the temperature fell to 15° C.

We may conclude from these experiments that sunlight is not a factor in producing autotomy in *Tubularia*.

With respect to darkness, data have been given already to show that this is not a potent factor. In the case of hydranths placed in the ice-chest, the individuals were not exposed to light

for more than a few moments during the day. Therefore, if the absence of illumination was a positive factor in inducing the process of autotomy, it most assuredly would have been made evident here. In another case, specimens were attached to weighted blocks of wood, as used in an earlier experiment, the block being covered with a tin case. These were lowered beneath a bridge over a tide-way to a depth of two fathoms. The individuals were therefore in almost total darkness. The temperature of the water at this depth was lower than that of the surface, owing to tide currents. The hydranths were retained for over two weeks, when the experiment was terminated.

3. Stems of *Tubularia* were tied to supports in running water of a temperature of about 20° C. The supports could be so arranged that the stems were inclined at any angle with respect to gravity. The hydranths were cut off by autotomy in three days at whatever angle the stems were placed. Inasmuch as this time corresponds to that in which the hydroids of colonies introduced into aquaria from the sea, lose their hydranths and since no decrease in the time of autotomy could be determined, to be correlated with the position of the hydroid with respect to gravity, the writer does not believe that this factor is operative in autotomy. Loeb, it will be recalled (*l. c.*), believed that gravity induced absorption in *Antennularia*. This case of Loeb's is the only one recorded in which gravity acts. When one recalls the fact that the stems of *Tubularia* are placed in any position with respect to the vertical in their natural habitat on rocks and piles, it would seem quite extraordinary that such effects of gravity should obtain in this species. Moreover, in strong currents, such as the tide which sweeps over the rocks of the Tide-mill at South Harpswell, Maine, the stems are subjected to a force which causes incessant change in their axes with respect to gravity.

4. To determine the effect of lack of oxygen, a liter of sea water was boiled for half an hour and cooled to 20° C. in an hermetically sealed vessel. Specimens of *Tubularia* were put into the water and the vessel, now exposed to the air, was placed in the ice-chest. Within two hours nearly every hydranth had fallen from the stems.

In another set of experiments, excess of oxygen was supplied by means of the Mast aërating device. The vessel containing the hydroid stems was placed in chipped ice and the aërating apparatus delivered bubbles of air to one corner of the dish. However, here, no lengthening of the period of retention of the hydranths could be determined when the temperature was permitted to rise to ordinary room temperature (21° C.). Therefore, while lack of oxygen causes autotomy to set in, an excess of the gas over and above that which forms normally the oxygen content of sea water at these temperatures does not prolong the period of retention of the hydranths; they behave as if they had been brought in from the sea and placed in an ordinary aquarium under ordinary conditions.

It will be recalled that the specimens in the fingerbowls which were kept in the ice-chest were not supplied with air artificially. Moreover, the water was not changed, so that the air which was over the surface of the water of the bowls was sufficient to supply the oxygen needed by the animals. The oxygen content of water kept under such conditions cannot vary to any great degree and the sea water, flowing over the animals in their natural habitats varies, as has been determined, comparatively little in the amount of air in solution. Moreover, the specimens which, on being introduced into the laboratory lose their hydranths, are kept, in the main, supplied with ever changing water in which the oxygen content is practically that of the surrounding sea, or even higher, owing to the condensation by the pumps. Therefore this factor cannot be of any considerable importance in causing autotomy.

5. Considerations were given to mechanical effects such as the swaying of hydranths on the stems in a strong current of water, rubbing of the stems against one another and mechanical shock induced by cutting off the hydranths from the stems.

With respect to the first factor, we know that in nature, in many cases, *Tubularia* grows where tide causes whirlpools and an ever changing direction in the currents passing over the hydroids and therefore, one would not look for this as a cause of autotomy. Experiments were performed to show that the factor is not operative, by placing stems, bearing healthy

hydranths in jets of water which caused an incessant waving of the hydranth on the stem. The hydranths were retained, under such conditions, as long as was compatible with the temperature and other external conditions. Abrasion was likewise made the question of another set of experiments and here, too, no effect could be correlated with the factor. With respect to mechanical shock, when the stems are removed from the colonies, data have already been given, for in the ice-chest experiments, where the hydranths were retained for a long time, the stems had been cut from the colony. Moreover, in those specimens which were placed on floats in the sea and where the hydranths did not fall off, the individuals had been cut from the stems. Therefore, mere cutting of the stems is not a factor in autotomy.

It will be seen that temperature seems to be the only consistent factor involved in the decapitation. When the temperature is kept at about 10° C. or 15° C., the hydranths are retained, regardless of any other factors with the one exception of lack of oxygen, which we believe to be inoperative except under wholly artificial conditions. When it is recalled that in the sea, *Tubularia* may be found throughout the summer[1] in latitudes where the temperature of the water does not rise above 18° C. or 20° C., while in lower latitudes[2] the occurrence of this species on the piles and rocks is correlated with the times of the year, spring and fall, when the water is cooler, we find sufficient data from natural sources to conclude that temperature is the potent factor in causing autotomy of the hydranths in colonies brought into the laboratory.

The case of *Hydra*, described by Greely[3] where the body becomes reduced to a mass of dissociated cells when the temperature is reduced to 4° C. and 6° C., seems to weigh against the present conclusions, but more recently Caroline McGill[4] has

[1] As at South Harpswell, Maine, where the temperature of the water seldom rises above 16° C.

[2] *E. g.*, Woods Hole, Mass. *Tubularia* disappears when the temperature of the water reaches 20° C. It reappears again for a time in the fall.

[3] Greely, "Further Studies on the Effects of Variations of Temperature on Animal Tissues," Scientific Papers.

[4] McGill, C., "The Effect of Low Temperature on *Hydra*," BIOL. BULL., Vol. 14, 78.

studied the histological changes occurring in *Hydra* subjected to reduced temperatures and is unable to corroborate Greely, but rather shows that a loss of water accompanies the lowering of the temperature, which causes the hydroids to become opaque, without degenerative changes in the cells.

Little success has been had with *Tubularia* in exhibition tanks. If some means could be devised for keeping the water in such tanks at a temperature, say of 15° C., the writer believes that *Tubularia* could be maintained in health, as in the sea.

The writer has studied *Tubularia* at the Bureau of Fisheries and the Marine Biological Laboratory, at Woods Hole and at the Harpswell Laboratory. To those in charge of these laboratories, Professors Sumner, Lillie and Neal and to Dr. Kingsley and Professor Lambert, the writer is indebted for much kindness. The work was started at the suggestion of Dr. T. H. Morgan and the writer is grateful to him for generous aid and criticism.

College of the City of New York,
December 15, 1908.

PRELIMINARY REPORT ON THE EARLY HISTORY OF THE EGG AND EMBRYO OF CERTAIN HYDROIDS.

CORA JIPSON BECKWITH.

Within the last few years some of the hydroids (*Pennaria, Clava leptostyla, Eudendrium, Tubularia crocea*) have been described as differing widely in their processes of maturation, fertilization, and early cleavage from what we have come to think of as typical. Other members of the same group (*Tubularia mesembryantheum, Clava squamata, Hydra, Gonothyrea, Æquorea, Tiara, Gonionemus*) have been described as perfectly typical. That certain of the hydroids should conform to the type and others not, seemed improbable. The fact of the low organization of the group which is used by Hargitt to account for such variation seemed hardly sufficient. On this account at the suggestion of Professors Morgan and Wilson, I undertook the reëxamination of two of the forms, *Pennaria* and *Clava leptostyla,* which varied most from the type, to discover if possible the relation between the aberrations described in these forms and the usual type. The results on *Pennaria* were worked out in the winter of 1907 at the Zoölogical Laboratory of Columbia University, on material collected at Woods Hole during the previous summer. Those on *Clava* were obtained in the summer and autumn of 1908.

A brief review of Hargitt's work on *Pennaria* and *Clava leptostyla* will be necessary to recall the points which I wished to clear up. In both these forms, according to his results, the processes of maturation and fertilization of the egg are very obscure and incapable of demonstration. The germinal vesicle just before the time that maturation should take place moves to the periphery of the egg where it loses its staining capacity, the nuclear membrane breaks down, and the nuclear substance becomes diffused throughout the egg, where it is no longer recognizable, due probably to some chemical or physical change in the egg. The

maturation and fertilization processes were supposed to take place while the chromatin is in this unstaining condition, the sperm having also lost its staining capacity after entering the egg. The first evidence of nuclei in the egg after the disappearance of the germinal vesicle was found in the appearance of "nuclear nests" or groups of small vesicles scattered throughout the egg, several such nests usually occurring in an unsegmented egg. In *Pennaria* not less than four such nests appear simultaneously indicating four centers of nuclear reconstruction. In *Clava,* as I understand the description, this condition of several nuclear groups occurs occasionally in an unsegmented egg. More often, however, eggs were found already segmented with a single resting nucleus in each cell which I take it were believed to arise as described for *Pennaria.* This reappearance Hargitt thinks is explicable on the ground that after fertilization the chemical or physical conditions again change so that the chromatin once more responds to stains. The chromatic material that was scattered at the time the nucleus disappeared collects again, forms vesicles which especially in *Pennaria* occur in groups or nests, each nest finally fusing into a single nucleus. Thus a syncytium arises without mitosis and with no apparent evidence of maturation or fertilization having taken place in the egg. In *Pennaria* after these nuclear groups are formed, nuclear proliferation is by mitosis. In *Clava leptostyla,* however, up to the sixteen-cell stage Hargitt describes nuclear proliferation by amitosis, later cleavages being mitotic.

The points in question accordingly are: (1) The nature of maturation and fertilization processes, (2) the formation of nuclei *de novo,* and (3) the *rôle* of amitosis and mitosis in early cleavages.

Pennaria and *Clava leptostyla* were preserved at Woods Hole where Hargitt obtained his material. Also the same killing fluids and stains were used so that the question of method is eliminated. Material was killed every hour of the day and night and every half hour in the early morning hours which proved to be the most important period.

I found in both *Pennaria* and *Clava leptostyla* that in material killed between the hours of 4 and 6 A. M., it was possible to

demonstrate the maturation processes and that they take place with the utmost exactness and in the typical manner, as reference to the figures will show. Fig. 1 shows the germinal vesicle of an egg of *Pennaria* in which the inner wall is breaking down, the nucleolus passing into the cytoplasm where it is lost, while the chromatin is grouping itself around the periphery of the nucleus to form the chromosomes. Figs. 2 and 11 are nearly corresponding stages of the first polar spindle in *Pennaria* and *Clava leptostyla* respectively. In both forms the chromosomes are evidently bipartite and the number is determined to be one half the somatic number. In the figure of *Pennaria* (which is in the late prophase) the spindle has not yet swung around into position. A comparison of Figs. 3 and 12 shows again practically identical conditions of the second polar spindle in the two forms. The first polar body lies outside the egg, the second polar spindle is in the late anaphase. I have found numerous intermediate stages. Figs. 4 and 13 show corresponding stages of the reconstructed egg nucleus with the polar bodies lying outside the egg.

Figs. 5 and 6 give two stages in the fertilization of *Pennaria*. The two-germ nuclei lying side by side at the periphery of the egg later move toward the center of the egg where they form the fusion nucleus at the ends of which astral radiations appear. The origin of the first cleavage spindle is not determined. For lack of proper stages the fusion of the two-germ nuclei has not been demonstrated with certainty in *Clava leptostyla*.

From this point forward the two forms differ slightly. In *Pennaria*, after the first cleavage the two nuclei are reconstructed by the formation of chromosomal vesicles as shown in Figs. 7 and 8. For some reason, possibly the rapidity of nuclear divisions, the chromosomal vesicles often fail to fuse into a single nucleus but give rise to a "nuclear nest" which subsequently gives rise directly to the chromosomes of the following cleavage figure. Fig. 9 shows two such vesicles passing on to a spindle, one vesicle already broken up into the individual chromosomes, the other still in the vesicular stage. Fig. 10 shows an equatorial view of such a group of vesicles, some of the chromosomes already forming an equatorial plate.

A shortening of the resting stage between the nuclear divisions,

it seems to me, might account for Hargitt's interpretation of the "nuclear nests." The rapidity of nuclear division is accompanied by slow cytoplasmic division so that the former constantly outruns the latter, the result being that an unsegmented egg often contains several such groups of vesicles or "nuclear nests."

In *Clava* the cytoplasmic cleavage does not lag so far behind the nuclear division, but in fact keeps pace with it. For this reason it was possible as shown by Figs. 14, 15, 16, 17, 18 and 19 to demonstrate the first cleavage spindle, and the successive passage of the two-cell stage into the four-cell, eight-cell and sixteen-cell stages. The nuclear reconstruction takes place here again by the formation of chromosomal vesicles; but *Clava* differs from *Pennaria* in that, as a rule, the vesicles all fuse into a single nucleus between successive cleavages So far as I am able to tell as yet, it seems probable that the cleavage in *Clava* is fairly regular.

Summary.

In the two hydroids (*Pennaria* and *Clava leptostyla*) under question, the maturation and fertilization processes take place in a perfectly typical fashion and form no exception to the general rule in this regard.

The conclusion that the "nuclear nests" indicate the formation of nuclei *de novo* is shown to be untenable. The occurrence of these nests is explained by the conditions of nuclear reconstruction after cleavage, the chromosomal vesicles failing to fuse between successive divisions in *Pennaria* and the cytoplasmic division lagging behind nuclear division gives a syncytium with several nuclear groups.

Maturation and the early cleavages take place by means of mitosis and not amitosis. No evidence whatever of amitotic division was found.

My results regarding the maturation and fertilization phenomena make it very probable that Hargitt's failure to observe these stages was due simply to the fact that the eggs were not obtained at the right time of day. In eggs collected at the proper time (4–6 A. M.) there is no difficulty in proving the typical stages of maturation and fertilization.

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Explanation of Plate I.

Pennaria. All drawings × 1,300.

Fig. 1. Early prophase of germinal vesicle of egg of *Pennaria;* the inner nuclear wall is breaking down; the nucleolus passing into the cytoplasm; the chromosomes forming.

Fig. 2. Later prophase of first polar spindle of egg of *Pennaria;* some of the chromosomes bipartite; some quadripartite. Reconstructed from two consecutive sections.

Fig. 3. Anaphase of second polar spindle of egg of *Pennaria.*

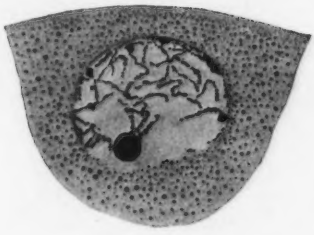
Fig. 4. Female germ nucleus and polar bodies in egg of *Pennaria;* chromatin is in fine reticulum.

Fig. 5. Fertilization of *Pennaria* egg; male and female germ nuclei at the periphery of the egg and of equal size. Reconstructed from three consecutive sections.

Fig. 6. Egg of *Pennaria* showing the fusion nucleus with astral radiations at either end of the nucleus.

Fig. 7. Telophase of 1st cleavage of egg of *Pennaria;* nuclear reconstruction by the formation of chromosomal vesicles.

Fig. 8. "Nuclear nests" formed by the partial fusion of the chromosomal vesicles lying at the ends of the spindle (probably second or third cleavage). *Pennaria.*



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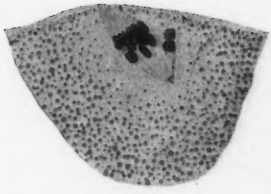

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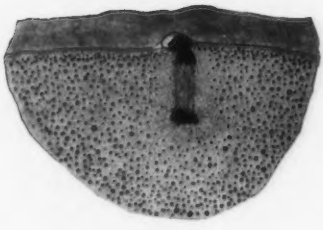

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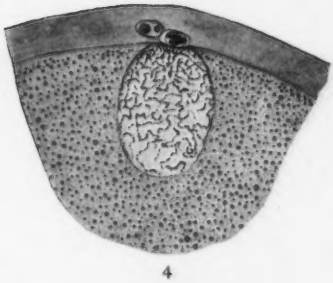

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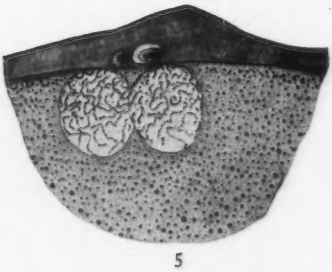

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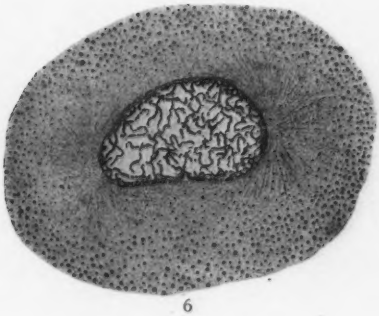

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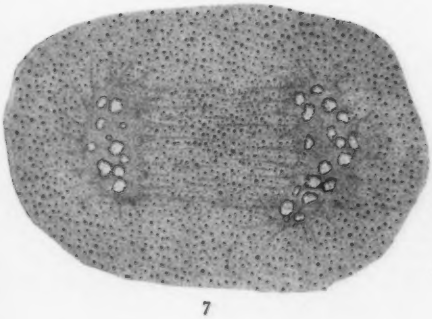

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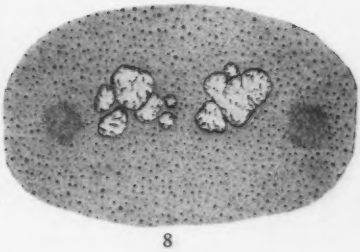

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Explanation of Plate II.

Pennaria. Figs. 9 and 10, × 1,300.

Clava leptostyla. Figs. 11 to 13, × 1,300; Fig. 14, × 350.

Fig. 9. Third or fourth cleavage spindle with "nuclear nest" passing on to it to form the equatorial plate, one vesicle broken up into chromosomes. *Pennaria.*

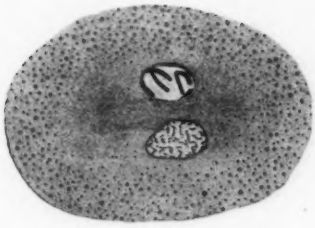
Fig. 10. Polar view of a cleavage spindle showing a "nuclear nest" as it is breaking up to form the equatorial plate, slightly later than the above, some of the chromosomes already free in the cytoplasm. *Pennaria.*

Fig. 11. Metaphase of first polar spindle of egg of *Clava leptostyla.*

Fig. 12. Anaphase of the second polar spindle of egg of *Clava leptostyla.*

Fig. 13. Female germ nucleus and polar bodies of egg of *Clava leptostyla.*

Fig. 14. Egg of *Clava leptostyla* showing the first cleavage spindle.



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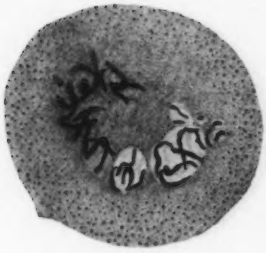

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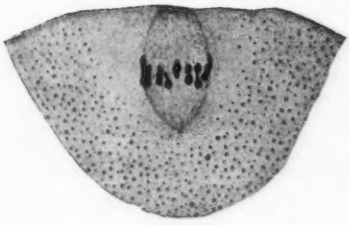

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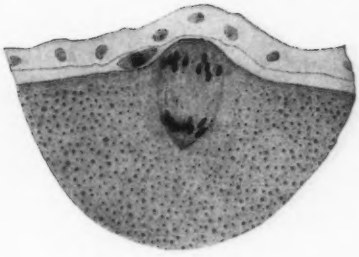

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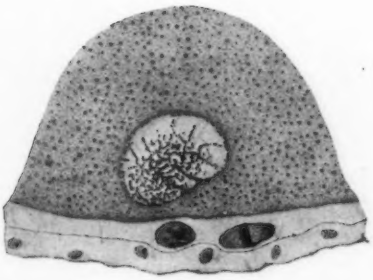

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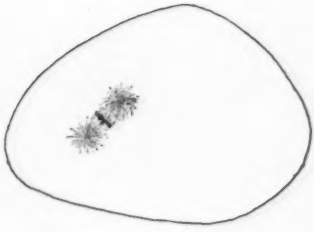

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EXPLANATION OF PLATE III.

Clava leptostyla. Figs. 15 to 19, × 350.

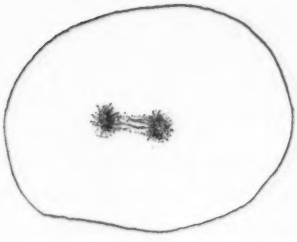
FIG. 15. Same, showing reconstruction of daughter nuclei by chromosomal vesicles.

FIG. 16. Two-cell stage of *Clava leptostyla* passing into the four-cell stage, second cleavage spindles showing.

FIG. 17. Four-cell stage of *Clava leptostyla* with resting nuclei.

FIG. 18. Four-cell stage of *Clava* passing into the eight-cell stage, spindles showing in two cells.

FIG. 19. Lateral view of eight-cell stage of *Clava* (five cells showing in section), some of the cells with spindles, some with resting nuclei.



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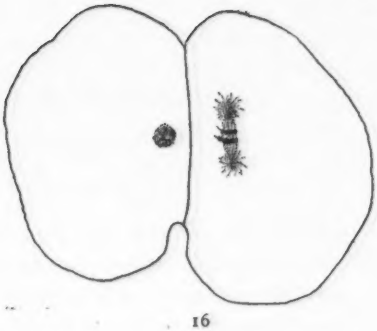

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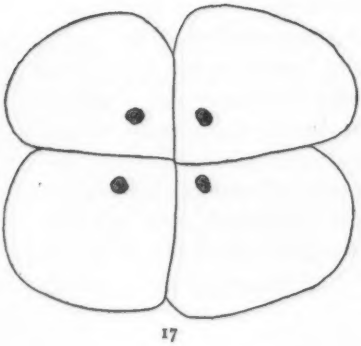

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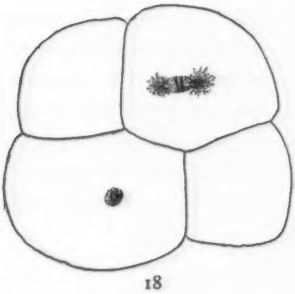

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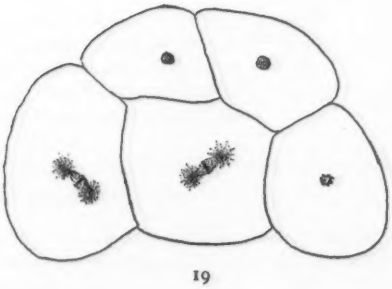

19

ON THE SEX OF HYBRID BIRDS.

MICHAEL F. GUYER,

UNIVERSITY OF CINCINNATI.

In a former paper[1] I have noted the difficulty of obtaining female hybrids from pigeons or doves of widely different parentage. Of the seven hybrid offspring of very distinct species then in hand, six were male. Since that time, through the courtesy of the Museum d'Histoire Naturelle in Paris and the Museum of Natural History in London, I have had the opportunity of examining a number of different hybrids in the family Phasianidæ and among them also I have found a remarkable predominance of males. In the following tabulations the sex of each hybrid, when known, and the parentage, is given, together with the date the individual was placed in the museum. It has been impossible to give the specific name always because a number of the specimens bore only the popular names. The letters placed after the year of accession indicate the respective locations of the specimen in question; thus, B = British Museum (Museum of Natural History); P = Museum d'Histoire Naturelle, Paris; C = Museum, University of Cincinnati.

GUINEA-FOWL × CHICKEN.

	Sex.	Date and Location.
Guinea-fowl × common fowl	= ?	1902 B.
Guinea-fowl × common fowl	= ♂	1899 B.
Pintade × Poule	= ?	1854 P.
Black Langshang Cock × Guinea-hen	= ♂	1903 C.
Black Langshang Cock × Guinea-hen	= ♂	1903 C.
Black Langshang Cock × Guinea-hen	= ♂	1903 C.
Black Langshang Cock × Guinea-hen	= ♂	1908 C.
Black Langshang Cock × Guinea-hen	= ♂	1909 C.

Thus, of eight guinea-chicken hybrids, the sex is known in six cases and it is invariably male.

[1] Guyer, M. F., "Spermatogenesis of Normal and of Hybrid Pigeons," Dissertation, University of Chicago, 1900. Also published as Bul. 22, University of Cincinnati, 1903.

PHEASANT × CHICKEN.

Chrysolophus pictus × Bantam fowl	= ♂	1890 B.
Phasianus colchicus × Game bantam	= ♂	1902 B.
Phasianus colchicus × Spanish fowl	= ?	1845 B.
Phasianus colchicus × Common fowl	= ♂	1884 B.
Phasianus colchicus × (Japanese long-tailed cock × common hen)	= ♂	1905 B.
Faison × Poule	= ♂	1851 P.
Faison × Poule	= ♂	1845 P.
Faison × Poule	= ♂	1836 P.
Faison × Poule	= ♂	1855 P.
Faison × Poule	= ♂	1813 P.
Faison × Poule	= ♂	1851 P.
Faison × Poule	= ♂	1851 P.
Faison × Poule	= ♂	1846 P.

It will be seen that of thirteen pheasant-chicken hybrids, the twelve of which the sex is recorded are all male.

PEAFOWL × CHICKEN.

Paon × Poule Cochinchinoise	= ♂	1907 P.
Paon × Poule Cochinchinoise	= ♂	1907 P

From the foregoing it will be observed that of the total of twenty-three hybrids from markedly different parentage (guinea × chicken, pheasant × chicken, and peafowl × chicken), each one of the twenty of which the sex is known is male.

PEAFOWL × PEAFOWL.

Pavo cristatus × *Pavo muticus*	= ♂	B.

PHEASANT × PHEASANT.

Chrysolophus pictus × *Phasianus reevesi*	= ♂	1887 B.
Hybrid *P. colchicus-reevesi* × *Gennæus nycthemerus*	= ?	B.
Chrysolophus pictus × *Phasianus colchicus*	= ♂	1855 B.
Hybrid *P. colchicus-reevesi* × *Gennæus nycthemerus*	= ♂	1904 B.
Gennæus horsfieldi × *Phasianus versicolor*	= ♂	1866 B.
Hybrid *P. reevesi-colchicus* × *Gennæus nycthemerus*	= ♂	1902 B.
Phasianus colchicus × *Gennæus nycthemerus*	= ♂	1902 B.
Hybrid *C. pictus-amherstiæ* × *Phasianus colchicus*	= ♂	1897 B.
Chrysolophus pictus × *Gennæus nycthemerus*	= ♂	1906 B.
Phasianus colchicus × *Chrysolophus pictus*	= ♂	1904 B.
Phasianus colchicus × *Gennæus melanotus*	= ♂	1865 B.
Phasianus colchicus × *Chrysolophus amherstiæ*	= ♂	1898 B.
Lophophorus impeyanus × *Euplocamus*[1] *melanotus*	= ?	1893 P.

[1] *Euplocamus* is a synonym of *Gennæus*.

[2] Presumably *C. pictus*.

Faisan doré[2] × Faisan commun[3]	= ♂	1842 P.
Faisan à collier[4] × Faisan argenté[5]	= ?	1886 P.
Faisan commun[3] × Faisan amherst[6]	= ?	1837 P.
Faisan doré[2] × Faisan ordinaire[3]	= ♀	1853 P.
Faisan commun[3] × Faisan argenté[5]	= ♂	1837 P.
Faisan commun[3] × Faisan argenté[5]	= ♂	1843 P.
Phasianus mongolicus × *Phasianus colchicus*	= ♂	1906 B.
Phasianus colchicus × *Phasianus reevesi*	= ♀	1904 B.
Phasianus colchicus × *Phasianus torquatus*	= ♂	1894 B.
Phasianus colchicus × *Phasianus reevesi*	= ♂	1894 B.
Chrysolophus amherstiæ × *Chrysolophus pictus*	= ♂	B.
Phasianus colchicus × *Phasianus reevesi*	= ♂	1897 B.
¾ *Chrysolophus amherstiæ* × ¼ *Chrysolophus pictus*	= ♂	1887 B.
Euplocamus[1] *swinhoii* × *Euplocamus nycthemerus*	= ♂	1882 P.
Euplocamus swinhoii × *Euplocamus nycthemerus*	= ♀	1875 P.
Euplocamus lineatus × *Euplocamus nycthemerus*	= ♂	1887 P.
Euplocamus lineatus × *Euplocamus nycthemerus*	= ♀	1878 P.
Euplocamus horsfieldi × *Euplocamus lineatus*	= ♂	1819 P.
Euplocamus horsfieldi × *Euplocamus lineatus*	= ♂	1869 P.
Faisan amherst[6] × Faisan doré[2]	= ?	1882 P.
Faisan amherst × Faisan doré	= ?	1902 P.
Faisan commun[3] × Faisan à collier[4]	= ?	1860 P.
Faisan commun × Faisan à collier	= ♂	1858 P.
Faisan commun × Faisan à collier	= ♂	1843 P.

Of a total of thirty-seven hybrid pheasants, nineteen have been from parents sufficiently widely separated to be ranked by systematists as separate genera or subgenera, and of these nineteen, fifteen were of known sex, namely, fourteen males and one female. Of the remaining eighteen there were twelve males, three females and three of which the sex was undetermined.

Thus of a grand total of sixty-one hybrids, the sex is known in fifty-one cases and among these there are only four females in all. Furthermore, three of these females were hybrids between species of the same genus, the other one, between species from genera not widely divergent. In hybrids between individuals of distantly related genera or between individuals from different subfamilies (*e. g.*, guinea × chicken) where the sex has been recorded it has been invariably male.

There are three possible sources of error in these data. In the

[3] Presumably *P. colchicus*.
[4] Presumably *P. torquatus*.
[5] Presumably *G. nycthemerus*.
[6] Presumably *C. amherstiæ*.

first place it is known that sterile females sometimes, although rarely, take on the male plumage, and it may be urged that there is no means of knowing certainly that the sex was determined beyond all doubt by opening the abdominal cavity and finding the testes. However, since the specimens had to be partially dissected before the skins could be mounted, it is reasonable to suppose that in the vast majority of cases the sex was thus accurately determined. The five guinea-chicken hybrids as well as the six dove and pigeon hybrids mentioned in my former paper were all dissected by me personally and consequently I am sure of their sex.

In the second place the objection may be raised that possibly the museums have preserved only the males, inasmuch as they make handsomer specimens and are not as similar in appearance as female pheasants. There is, of course, a possibility of this, especially in the case of hybrid pheasants from closely related species. Hybrids from widely different parents are so rare, however, that there is every probability that if there had been females they as well as the males would have been preserved. As a matter of fact, the few female pheasant hybrids that I have been able to find in museums are not similar in appearance nor do they resemble the males. As *hybrids* they are as interesting in every way as the males and it seems probable, therefore, that had there been more of them they would have been preserved. When due allowance is made for all errors the facts still indicate that there is a marked tendency for hybrids, especially those from widely separated parents, to be male.

Lastly, there is the remote possibility that there has been a greater mortality among the females in early life. In the few cases (guinea-chicken hybrids and various pigeon hybrids) of which I have data regarding the number of eggs laid and the history of the young, there is no evidence of such mortality.

It may be noted in passing that in the collections of the British Museum there is to be seen a hybrid between individuals of two different families, namely, a penelope (Family Cracidæ) and the common fowl (Family Phasianidæ). This hybrid resembles more the fowl than the penelope. Unfortunately the sex is not recorded.

In looking over the literature of the subject to see if anything had been recorded concerning the sex of hybrids outside the group Phasianidæ, I found that in general little attention had been paid to it. Some mention is made of the sex of hybrids in Suchetet's[1] voluminous work on hybrid birds. In speaking (p. cxvii) of hybrids and mongrels, he asserts that among the former he believes there are more males than females, and he cites various authorities in substantiation of his belief. Thus, according to data collected by Buffon, there are more male than female mules and Buffon asserts, furthermore, that among hybrid birds the number of males exceeds very much that of females. Suchetet cites the following figures from Buffon: the proportion of males to females in hybrids between the he-goat and the ewe are 7 to 2; between the dog and the wolf, 3 to 1; between the goldfinch and the canary, 16 to 3. Suchetet cites still further examples from other authorities, but he seems not to have gone over his own extensive notes on hybrids with this question of sex in mind. For example, on pages cxxi–cxxxiv he gives a statement in tabular form of data collected from some eighty-five public and private museums concerning in all 234[2] specimens of hybrids between wild birds (*i. e.*, not domesticated) or of forms reputed to be such hybrids. Since in many cases the sex of these hybrids has been given, I have gone through the tables and arranged the birds according to sex as far as it is indicated, with the following results:

Of hybrids between species bearing the same generic name there are in all 124, of which 72 were male, 18 female and 34 of undetermined sex. The remaining 110 hybrids were between individuals bearing different generic names and of these 74 were male, 13 were female and 23 were of undetermined sex. Thus it will be seen that the males far outnumber the females in each case. Furthermore, this would remain true in the proportion of about 3 to 2, even should it be counted that all those of *undetermined* sex were female!

In his later amplifications of this list he discusses (p. 507) 48

[1] Suchetet, André, "Des Hybrides à L'Etat Sauvage; Oiseaux," Vol. I., 1896, Lille. A large volume of over 1,000 pages.

[2] Suchetet states the total as 236 but he has made an error of 2 in his addition on page cxxxii.

additional hybrids between *Tetrao tetrix* and *Tetrao urogallus*, of which 40 are male and 8 female. Again, page 573, he lists 20 hybrids of *Lagopus albus* and *Tetrao tetrix*, of which 13 are male and seven are female.

As to the general bearing of these facts upon any one of the numerous theories of sex-determination, the writer does not feel disposed to dogmatize, although certain suggestions present themselves. For a general and unbiased statement of our present knowledge regarding the question of sex-determination, the reader may consult the recent publications of Thomson[1] or of Morgan.[2]

Both of these writers agree that when all the evidence is considered it does not seem improbable that the conditions which regulate the development of sex may be different in different kinds of animals. Regarding the sex-determining influence of nutrition and temperature, either directly on the developing organism or through its parents, Thomson points out that while the evidence in any given case is inconclusive, still when all the cases are taken together, "they have a certain cumulative suggestiveness which would warrant further experiment—particularly as regards the lower animals and the indirect influence on offspring through the parents" (1908, p. 490).

In general, where the experiments tend to show that nutrition is a factor after the period of fertilization, it has been the production of females that was supposedly favored by such increased nutrition; the question being apparently one of increased constructive metabolism. It would follow that anything tending to retard or hold at a low ebb the constructive phases of metabolism, especially during early embryogeny, would be inimical to the production of females. Now in the case of hybrids, and particularly those from widely separated parents, there would in all probability be more or less default in the metabolic processes because of the incompatibilities which must necessarily exist between two germ-plasms so dissimilar. It seems not improbable, therefore, that this might be the determining factor in the production of an excess of males in the case of such hybrids.

[1] Thomson, J. Arthur, "Heredity," London, 1908.

[2] Morgan, T. H., "Experimental Zoology," New York and London, 1907.

THE FEMALE CHROMOSOME GROUPS IN SYROMASTES AND PYRROCHORIS.

EDMUND B. WILSON.

The conditions seen in *Syromastes marginatus* L. are of interest on account of the light that they throw on those observed by Morgan in Phylloxera, as reported by him at the December meeting of the American Society of Zoölogists, and recently published in the issue of *Science* for Feb. 5, 1909. In both forms the "accessory" chromosome is not a single but a double body, and the female chromosome-groups contain two more chromosomes than the male.

A reëxamination of the spermatogenesis of *Syromastes*[1] led to a confirmation of Gross's result that the spermatogonial number is even (22), and that the "accessory" chromosome is formed by the union of two chromosomes that are separate in the spermatogonia. This double element divides equationally in the first spermatocyte-division but passes undivided to one pole in the second, so that half the spermatozoa receive two more chromosomes (12) than the other half (10). This led me to the inference that the female somatic groups should have two more chromosomes than the male—*i. e.*, 24 instead of 22, as had been described by Gross. I had at that time no female material, but through the kindness of Professor Boveri have since obtained an abundant supply of the ovaries. Examination of this material demonstrated the correctness of my earlier inference. A considerable number of ovaries have been sectioned, many of which contain numerous and very fine division-figures, showing the chromosomes with great clearness. Whenever a good view of the equatorial plate can be obtained, 24 chromosomes are unmistakably seen to be present, as is clearly shown in photographs. Two figures (Fig. 1, *c*, *d*) are appended, both of which were drawn upon enlarged photographs by the method described in my fourth "Study." *Syromastes* is an exceptionally favorable form

[1] "Studies on Chromosomes, IV.," *Journ. Exp. Zool.*, VI., 1, 1909.

for study, and the diagrammatic clearness with which the chromosomes appear in many of the sections precludes, I think, the possibility of error in respect to the number.

In my description of the male groups I emphasized the fact that the two components of the "accessory" are of slightly un-

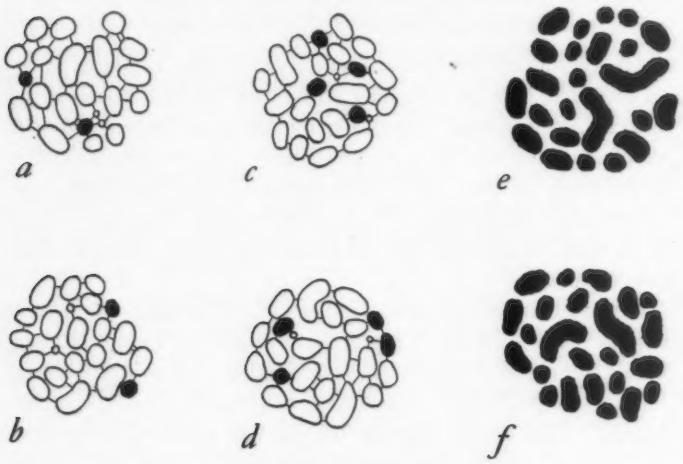

FIG. 1. *a, b, Syromastes marginatus* L., spermatogonial chromosome-groups; *c, d,* ovarian groups of the same; *e, f, Pyrrochoris apterus* L., ovarian chromosome-groups.

equal size (as is shown in the photographs accompanying the paper), and that they are recognizable in the spermatogonial groups as two separate chromosomes which are the second and third smallest of all the chromosomes. In the female groups each of these chromosomes is represented by a corresponding pair (black in the figures). In most of the ovarian groups the smaller two are readily recognizable, and in some cases, though not always, this is also true of the larger pair. The numerical and size-relations are such as to show that after maturation the egg must contain one member of each of these pairs. Though nothing is directly known of the maturation-process in the female, it may be inferred with probability that in synapsis the two larger and the two smaller of these pairs unite to form two corresponding bivalents, which may be designated as *aa* and *bb* (*II* and *ii*.

in the terminology of my former paper). By the subsequent disjunction of each of these pairs the mature egg receives *a* and *b* in addition to 10 other chromosomes. Fertilization of the egg by a spermatozoön containing the "accessory" $(a + b)$ will therefore give the characteristic female group $(a, b, a, b + 20)$, while fertilization by one that lacks $a + b$ will give the male group $(a, b + 20)$. For the sake of comparison two of the spermatogonial groups are reproduced in Fig. 1, *a*, *b*. In the figures of both sexes the chromosomes identified as *a* and *b* are made black. The essential relations in both sexes are shown in the diagram,

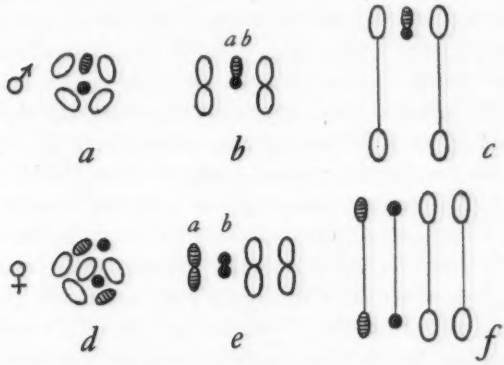

FIG. 2. Diagrams of maturation in *Syromastes*. *a*, spermatogonial chromosomes (actual number 22); *b*, *c*, spermatocyte-division; *d*, ovarian chromosomes (actual number 24); *e*, *f*, maturation division (inferred).

Fig. 2, in which *a* is cross-barred, *b* black, and the other chromosomes (of which only four are represented) are in outline.

It is evident that, except for the different total number of chromosomes in the two species, these phenomena are essentially similar to those seen in *Phylloxera fallax*, which likewise has two "accessories" and two more chromosomes in the female somatic groups (12) than in the male (10). In *Phylloxera caryœcaulis* the two "accessories" are of unequal size, as in *Syromastes*, but the phenomena are complicated by the fact that these two chromosomes are often united in the somatic groups of the male, and are apparently always thus united in those of the female. In the male they are always united at the time of the spermatocyte-

divisions to form the "accessory," which is in reality double, like that of *Syromastes,* and sometimes separates into its two components as it moves to the pole. Morgan therefore concludes that the true numbers of the chromosomes in the two sexes of this species are eight and six respectively, though the female seems to show but six and the male either five or six (according as the two "accessories" are united or separate).

The point to which I would call attention is the similarity between *Phylloxera caryæcaulis* and *Syromastes* in respect to the mode of synapsis. In the spermatogenesis of both forms the two unequal "accessories" unite in synapsis (if the process can properly be so called) to form the bivalent *ab,* consisting of two unequal components, which passes into the female-producing spermatozoa. In the maturation of the male-producing egg of *Phylloxera,* however (and apparently the same must be true of the sexual eggs), a different process takes place, the two larger and the two smaller components uniting to form the bivalents *aa* and *bb,* again exactly as there is reason to conclude in the case of the egg of *Syromastes.* In *Phylloxera,* as Morgan points out, this involves a redistribution of the four chromosomes, since in the somatic groups they are united to form *ab* and *ab,* but recombine at the maturation period to form *aa* and *bb.* This remarkable redistribution, I think, loses much of its anomalous character on comparison with the facts in *Syromastes,* where *a* and *b* are always separate in the somatic groups.

These facts, together with those determined by Payne in *Fitchia* and other forms (now in press in this journal) and my own earlier ones on *Thyanta,*[1] which shows essentially the same conditions as in *Fitchia,* lead me to a somewhat different interpretation of the "accessory" chromosome in *Syromastes* from that given in my fourth "Study." In that paper I adopted the conclusion that the two components of the bivalent "accessory" were identical respectively with the large and small "idiochromosomes" of such forms as *Metapodius* or *Lygæus.* I did not then see that all the facts are equally consistent with the view that these two components, *taken together,* represent the single odd

[1] Reported at the meeting of the American Society of Zoölogists in December, 1906, but still unpublished.

chromosome of *Anasa, Protenor* and other similar forms, and that the small idiochromosome has disappeared. Payne discovered that in the reduvioids, where a single small idiochromosome or "Y-element"[2] is always present, the large idiochromosome is in some species a single chromosome (*Diplocodus*), in others is represented by two (*Fitchia, Conorhinus*) or three chromosomes (*Prionidus*), and in the galgulid genus *Gelastocoris* (*Galgulus*) by four chromosomes, which behave in maturation as a single unit (X-element) that is obviously comparable to a single large idiochromosome in its relation to sex-production. Payne concludes, with great probability, that the double or multiple X-element in these forms has arisen by the separation of an originally single large idiochromosome (such as still exists in related species) into two or more components. In *Conorhinus,* where the X-element is double, the two components are unequal in size, and by the disappearance of the Y-element a condition would arise closely similar to that seen in *Syromastes.*

Whether such has been the actual mode or origin in *Syromastes* or not, it seems probable that here too the double "accessory" was originally a single chromosome that has separated into two parts, which still act as a unit in the maturation divisions and retain the same relation to sex-production as the original one. *Phylloxera caryæcaulis* may plausibly be regarded as in process of transition from the condition in which a single "accessory" chromosome is present (as appears to be the case in the aphids) to one in which it has separated into two parts, as in *Syromastes.*

I will add a brief account of the female groups in *Pyrrochoris apterus* L., material for which has also been obtained through Professor Boveri. A reëxamination of the male groups (Wilson, Study IV.) showed the spermatogonial number to be 23, including a single unpaired idiochromosome ("accessory" chromosome) which is at once recognizable from the fact that it is nearly twice the size of any of the other chromosomes. This passes into half the spermatozoa, which receive 12 chromosomes, while the others receive but 11, as was originally described by Henking. The

[2] In a recent general discussion I have used the terms "X-element" and "Y-element" to designate respectively the large and small idiochromosomes, or their homologues, whether they consist of a single chromosome or of more than one. See *Science,* XXIX., 732, January 8, 1909.

female groups clearly show 24 chromosomes, of which two are of the same relative size as the unpaired one of the male. Two of the ovarian groups, typical of many others observed, are shown in Fig. 1, *e, f*. These facts show that *Pyrrochoris* conforms to the ordinary type in which the male has an odd chromosome, as in *Anasa, Protenor, Alydus, Largus* and many others.

COLUMBIA UNIVERSITY,
January 23, 1909.

OBSERVATIONS ON THE GERM CELLS OF HYDRA.

GEO. W. TANNREUTHER.

The germ cells of hydra, which pass through stages comparable to those of higher animals, possess some peculiarities that have not been previously mentioned. Some investigators advocate the specificity of the germ cells, while others claim that the sex cells are the immediate derivatives of the ordinary interstitial cells.

Kleinenberg (4), Hertwig (3), Brauer (1) and others consider the sex cells as interstitial in origin and state that the egg first becomes recognizable after the ovary has begun to develop. Downing (2) claims that there is continuity of germ plasm in the sense of a specific line of germ cells, that the egg (*Hydra fusca*) is always present even before the interstitial cells begin to form the ovary and that the egg may grow rapidly and take the initiative in its formation. He furthermore believes that the egg is recognizable as such in the adult hydra and in general that in some stage in the embryonic development certain cells are stamped with sex characters, so that they and their progeny form the sex cells distinct throughout the life of the individual.

A careful examination of sections from *Hydra* sp.? (Brauer) gives pretty conclusive evidence that not only the egg but the sperm as well is interstitial in origin. There can be no question in case of the sperm, as the different stages in development can readily be traced from the interstitial cells to the mature sperm. Furthermore, the progenitors of the spermatozoa have no special characters by which they can be recognized as germ cells. The cells that give rise to the eggs are interstitial in position and can be distinguished in the adult hydra from the ordinary interstitial cells by their large nucleus, nucleolus and abundance of chromatin, even before the growth of the ovary begins, as Downing states. This is especially true during the breeding season. If these sex cells could be distinguished during the budding season as well, it would at least suggest specificity of the germ cells.

In the diœcious form *Hydra* sp.? (Brauer) the egg cells can-

not be distinguished from the ordinary interstitial cells except during the period of sexual reproduction. Fig. 1, *a* and *b*, represents two adjacent eggs at the stage of development when they first become recognizable. They have begun to enlarge and can readily be distinguished from the adjoining cells by their size and vacuoles next the nucleus. The above figure was taken from an adult hydra in which six eggs were present in different stages of development, ranging from the interstitial cells to the undisputed egg. These eggs are isolated or found in groups. When two or more are found adjacent the cell walls become dissolved and one persists as the developing egg (Tannreuther). In a few instances observed two of these adjacent cells persisted and gave rise to two mature eggs. These cases, however, are extremely rare in proportion to the number of eggs produced.

The above results do not warrant the view that there is continuity of germ plasm in *Hydra*. Until sex cells distinct from the somatic can be traced through successive generations, we have no positive evidence of such a continuity.

The different generations of the sex cells of *Hydra* are distinct and can readily be recognized. In the formation of the egg there is a distinct growth period. Reduction occurs at the end of the growth period just before the first polar body is formed. The polar bodies remain attached after cleavage has begun, by means of a single cytoplasmic thread. The first polar body is larger than the second.

The spermaries are composed of an indefinite number of cysts. The individual cysts originate from a single or several interstitial cells. Fig. 2 represents a longitudinal section of a single cyst containing spermatogonia, which have originated from a single interstitial cell. After the spermatogonia have divided a few times (the number of divisions varies in different individuals) those found at the distal end of the cyst become transformed into the spermatocytes of the first generation without growth. In Fig. 3 the spermatocytes of the first generation are recognized by their dense chromatin mass. Different stages of development are found in a single cyst, ranging from the spermatocytes of the first generation to mature sperm, as shown in Fig. 4. The successive zones of the developing sperm are distinct. The nuclei

at the extreme proximal end of the cyst cease dividing and become the nuclei of new interstitial cells after the spermaries have disappeared.

My observations on the nuclear changes in the different stages of development confirm those of previous investigators. Reduction occurs after the last spermatogonial division just before the spermatocytes of the first generation divide. In the division of the nuclei of the spermatocytes of the first and second generations, the cell wall remains intact and produces a multinucleate cell (Figs. 5–7), and give rise to four sperm within a common

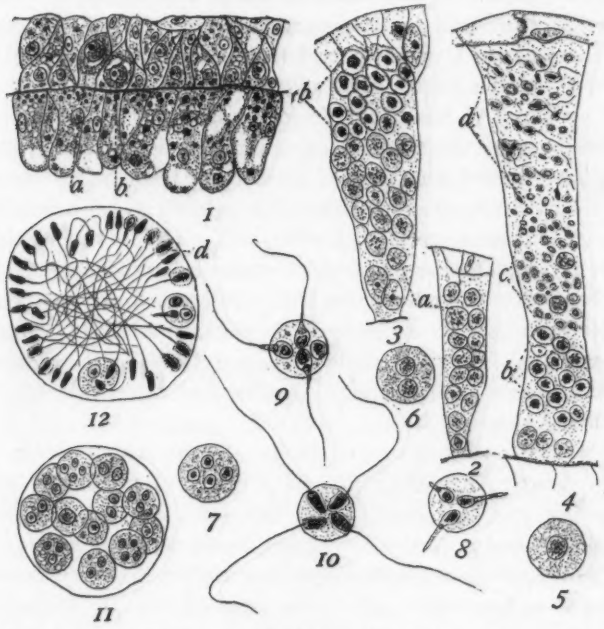

FIG. 1. Longitudinal section showing two developing eggs. *a* and *b*, eggs.

FIG. 2. Longitudinal section of a single cyst.

FIG. 3. Section of cyst little later than preceding. *a*, spermatogonia; *b*, spermatocytes of the first generation.

FIG. 4. Longitudinal section of cyst showing maximum development. *c*, spermatocytes of the first and second generations; *d*, mature sperm.

FIGS. 5–7. Formation of spermatids within common vesicle.

FIGS. 8–10. Developing sperm. Figs. 5–10, from living cells.

FIGS. 11 AND 12. Cysts removed from living spermary.

vesicle. The vesicular wall is very thin and the four sperm pass through it when mature and become free within the distal end of the cyst (Fig. 4). The mature sperm are very active within the cyst and finally escape to the exterior through a small temporary opening of the spermary. After the mature sperm have all escaped, the opening closes until more sperm mature. When the spermaries are extremely large, this process continues from forty to fifty hours.

In order to show the four sperm within a common vesicle, it is necessary to dissect the living spermary apart, as the sperm always escape from the common vesicle before passing to the exterior. The individual cysts when separated become more spherical (Figs. 11 and 12), and the different stage of development in the living sperm can easily be distinguished.

Kleinenberg in his description of the sperm in *Hydra viridis* definitely states that the sperm are formed from interstitial cells that have divided a number of times; ultimately the nucleus of the cell (the spermatocyte of the first order) disintegrates while the cell substance becomes granular, and in place of the nucleus there appears from one to four refractive bolies, which give rise to the sperm. The refractive bodies referred to beyond doubt result from the two divisions of the nucleus, which he thought disintegrated. The four nuclei (spermatids) within the common vesicle do have the appearance of refractive bodies, especially in the living material.

Korotneff (5) gave similar results. He states that the sperm form directly from the nuclei of a multinucleate mother cell. Downing (2) does not mention this interesting phenomenon, which is found in *Hydra viridis* and *Hydra* sp.? (Brauer).

The mature sperm possess extreme vitality and may remain active from one to three days after escaping from the spermary. The mature sperm of any individual spermary possess about the same degree of fertility. No degenerating spermatogonia were found.

In the moncecious form *Hydra viridis* the spermaries become mature before the ovaries. Occasionally sperm and ova on one individual would ripen at the same time, making self-fertilization possible. In order to prove that self-fertilization did occur, indi-

viduals with both spermaries and ovaries at the same stage of development were isolated and placed in distilled water for one hour, in order to kill any mature sperm that might adhere from other individuals. The *Hydra* were then placed separately in water free from sperm. The spermaries and ovaries matured and self-fertilization took place. The cleavage was normal.

Summary.

1. *Hydra* sp.? (Brauer) does not show continuity of the germ plasm.

2. The eggs can be distinguished from the interstitial cells in the adult *Hydra* before the ovary is formed. This is especially true during the breeding season.

3. The different generations in the formation of the germ cells are comparable to those of higher animals.

4. In the division of the spermatocytes of the first and second generations the cell wall remains intact and the four spermatids are formed within a common vesicle, each producing a mature sperm.

5. Self-fertilization occurs in *Hydra viridis*.

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BUDDING IN HYDRA.

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Budding in hydra has received the attention of only a few investigators. Our principal information as to the origin of the bud is derived from Lang's[1] account. He describes the bud as beginning by an increase in volume and division of the interstitial cells. After the ectoderm becomes thickened, the mesoglea disappears and the cells pass from the ectoderm into the endoderm. This process continues until the ectoderm becomes reduced to its normal thickness. The mesoglea then reforms and a cavity appears in the thickened endoderm, which becomes the enteron of the new individual.

The main object of the present paper will be to give a brief account of the origin and development of the buds in hydra, more especially their manner of growth and what cells contribute most to their rapid formation.

The species studied, *Hydra viridis* and *Hydra* sp.? (Brauer) differ somewhat from Lang's account. The mesoglea does not disappear and the ectodermal cells do not pass into the endoderm. The bud, however, begins by an increase in volume and division of the interstitial cells. After they have increased once or twice in volume, as shown in Fig. 1, there is a slight outbulging of the ectoderm, which is scarcely perceptible. Fig. 2 represents the condition of the interstitial, ectodermal and endodermal cells in the origin of the bud as they appear more highly magnified. A few mitotic figures are visible. No amitotic divisions were observed. The endodermal cells contain numerous food particles, which may pass intact through the mesoglea into the ectoderm. The cells directly concerned in the formation of the bud are well supplied with food, while the remaining cells of the parent hydra show a scarcity. Many of the endodermal cells in the distal half of the hydra have a glandular appearance and are most active in

[1] Lang, Albert, "Uber die Knospung bei *Hydra* und einigen Hydropolypen," *Z. wiss. Zool.*, Vol. 54, 1892.

the secretion of the digestive fluid, which aids in the breaking up of the food in the enteron, while those endodermal cells in the region of growth, "the formation of buds and sexual organs," are the most active in ingesting the partly digested food from the enteron and preparing it for diffusion into the ectodermal cells.

The ectoderm and endoderm in the early formation of the bud, which are quite uniform, soon become differentiated into two dis-

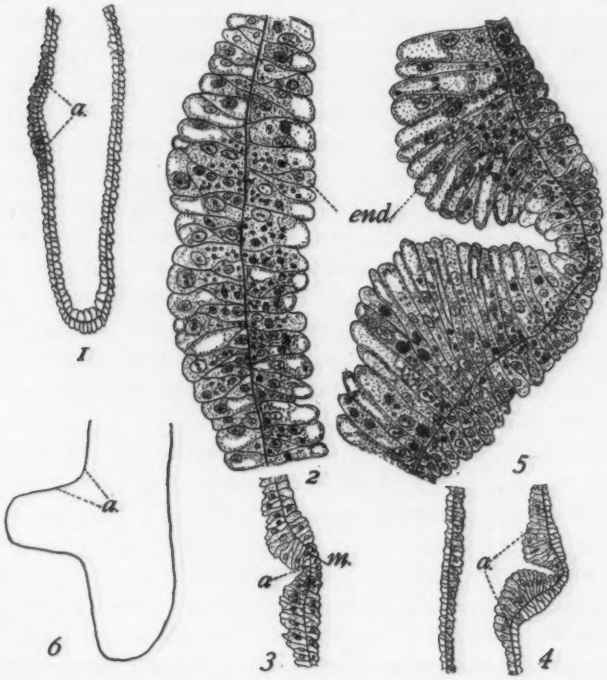

FIG. 1. Longitudinal section of hydra showing the origin of bud at *a*.

FIG. 2. Part of preceding figure at *a*. × 80.

FIG. 3. Longitudinal section through wall of hydra showing curvature of mesoglea and beginning of enteron in forming bud. *m*, mesoglea; *a*, enteron.

FIG. 4. Stage of development a little later than preceding. *a*, the point of junction between parent and bud.

FIG. 5. A portion of preceding figure at *a*, which includes the entire area in the formation of bud. *end.*, endoderm. × 90.

FIG. 6. Diagram of a longitudinal section of parent hydra with bud. *a*, active growing region in formation of bud.

tinct regions. The cells more centrally placed (Fig. 3, *a*), which correspond to the distal end of bud, become inactive, while those on either side of the central region continue active, divide rapidly and contribute almost entirely to the rapid growth of the bud. There is a slight curvature in the mesoglea (Fig. 3, *m*), and the enteron of the new individual becomes apparent. In a stage little later than the preceding (Fig. 4), the relation of bud to parent and the condition of cells is more plainly represented. Fig. 5, a portion of Fig. 4 at *a*, represents the entire area that contributes directly to the growth of the bud. The cells at the apex of the forming bud are small. Those on either side are larger and more active in the process of division and growth. Their contents is very similar, showing an abundance of food material and cytoplasmic granules.

The ectoderm and endoderm at the junction of the parent and bud (Fig. 7) divide very rapidly and become the most active growing region in the production of the new individual. The remaining cells of the bud seldom divide. The formation of the tentacles is similar to that of the buds. The cells corresponding to their basal ends take the most active part in their growth. The mouth is formed at the distal end of the bud by a breaking through of the ectoderm.

The rate of growth of the bud is determined by the amount of food present. In an active feeding hydra, the buds are often completely formed in thirty to forty hours. While in those hydras with a moderate supply of food the buds grow very slowly and may require four or five weeks or even more time for their complete development. In the latter instance after the buds are nearly formed, they will be absorbed in the absence of food. In the process of absorption the cell walls of the bud become imperceptible and the cell contents presents the appearance of a complete syncytium. When the buds are nearly absorbed, if the hydra is again supplied with food, the buds very seldom reform. In a few instances the buds were neither absorbed nor reformed, but remained attached to the parent as permanent individuals. Tentacles were formed later.

When the buds have reached their complete development the ectodermal cells at the proximal end undergo a rapid change

(Fig. 8, *ect*). They become more narrow, elongated and present the appearance of glandular cells. They have the power to

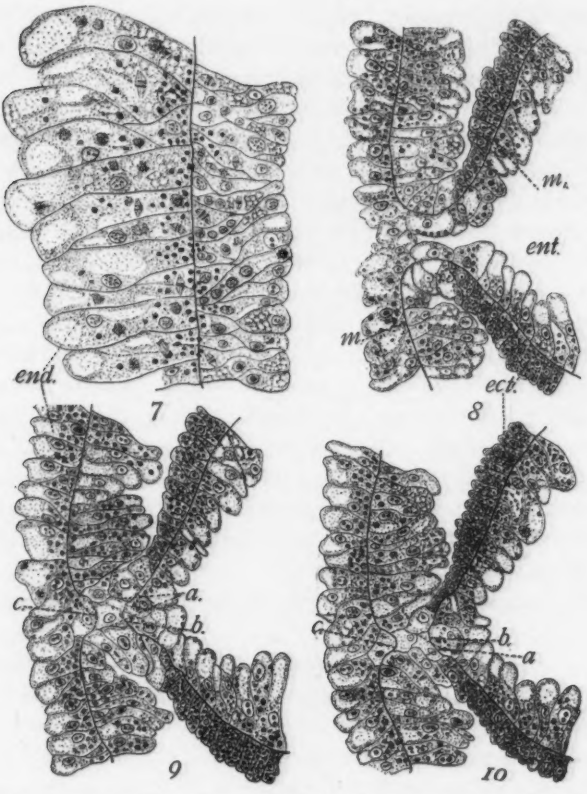

FIG. 7. Longitudinal section at junction of parent hydra and bud, taken at *a* in Fig. 6. *end.*, endoderm. × 100.

FIG. 8. Longitudinal section through base of mature bud and wall of parent hydra. m_1, mesoglea of bud; *m*, mesoglea of parent; *ect.*, glandular ectoderm; *ent.*, enteron of bud.

FIG. 9. Longitudinal section showing change of mesoglea in separation of parent and bud at *a*, and formation of new mesoglea at *b* and *c*. The endodermal cells at proximal end of bud have united. *a*, mesoglea between bud and parent becoming thinner; *b*, new mesoglea of bud; *c*, new mesoglea of parent.

FIG. 10. Longitudinal section showing completion of mesoglea at *b* and *c* and persistence of old mesoglea at *a*.

secrete a sticky substance before the bud becomes separated from the parent.

The first step in the process of separation of bud from parent occurs in the mesoglea. Fig. 8 represents the condition of mesoglea and cells before separation begins. The mesoglea, which connects the bud with parent, is uniform throughout. But almost immediately it becomes thinner and thinner until it is indistinguishable from the ordinary cell wall (Fig. 9, *a*). The enteron leading from the parent to the bud becomes discontinuous by the union of the endodermal cells. New mesoglea is now formed at the extreme basal end of the bud (Fig. 9, *b*) and at the point of former union with the parent at *c*. The mesoglea, which is more of a gelatinous nature, increases in thickness by means of secretion from the endodermal cells and soon reaches its normal condition (Fig. 10, *b* and *c*). After the formation of the mesoglea is complete, the bud remains attached to the parent by a few ectodermal and endodermal cells, as shown in Fig. 10. The former connecting mesoglea is represented by the dotted lines at *a*. The cells between the dotted lines, which are endodermal, become external to the newly formed mesoglea and take the position of ectoderm. Whether these cells persist or not and function as ectoderm is difficult to say, as there is no possible means of following them in the process of separation of bud from parent.

Summary.

1. The initial step in the formation of bud in hydra is found in the interstitial cells.

2. The most active region of growth in the formation of the bud is found at the junction of the forming bud and parent, where the cells divide very rapidly and contribute almost entirely to its growth.

3. When the bud is nearly formed the ectodermal cells in the basal region become transformed into granular glandular cells, which later secrete a glutinous substance for attachment of hydra.

4. The rate of growth in buds is controlled by the amount of food present. Starvation after buds are nearly complete often causes their complete absorption by parent hydra.

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